Developing an *in vitro* Model for Metastatic Breast Cancer Using Multicellular Tumor Spheroids

Every year, over 200,000 people are diagnosed with breast cancer making it one of the most common forms of cancer. Along with being the one of the most common cancers, breast cancer can also become one of the most deadly when it becomes metastatic. In fact, about 90% of cancer-related deaths are due to metastasis. This makes having in vitro models to study breast cancer beneficial for studying tumorigenesis and metastasis and for identifying novel therapeutic targets. In our lab, my project centered on developing a protocol to generate multicellular tumor spheroids (MCTS) composed of breast cancer cell lines that mimic key physiologic conditions and closely represent in vivo tumor architectures. We began this work by first conducting a primary literature search into methods that previous researchers have used to create MCTS. By adapting existing methods, we were able to create protocols to generate MCTS for four breast cancer cell lines: MDA-MB-231, BT-549, Hs578T, and MCF-7. Because each cell line is characterized by unique genotypic and phenotypic properties, we found differences in spheroid formation across the cell lines under the same conditions. Therefore, we varied experimental parameters to achieve optimal spheroid formation, which included altering the number of cells added per spheroid, the length of time for spheroid growth, and the type and concentration of extracellular matrix proteins included in the growth medium. We conducted over ten different experiments and gathered data on spheroid growth and compactness over a seven-day period. Images of spheroids were captured every 24 hours and image processing tools were used to analyze individual spheroids for diameter, compactness, and overall uniformity. The results from this work suggest that using approximately 10,000 cells per spheroid, a growth period of 4-5

days, and including 2.5% Matrigel in the growth medium reliably produced large (> 700 μm) and compact MCTS for the tested cell lines.

The objective of this research project was to devise a protocol to generate MCTS for various breast cancer cell lines to use as an *in vitro* model of tumor progression. MCTS with diameters greater than 500 μm have been shown to develop gradients in oxygen and nutrients, leading them to mimic features of in vivo tumors, such as a necrotic core and proliferative outer shell. Although each cell line posed a unique set of challenges in generating large and compact spheroids, we were able to succeed in developing a robust protocol to generate MCTS for each cell line that were all greater than 500 μm. We were also able to repeat the procedure several times to confirm its repeatability. Using the protocol developed through this project, our next step will be to perform histological analysis of individual MCTS to assess for markers of cell death and proliferation to confirm the development of a necrotic core and proliferative outer shell. This work will be carried out with the help of the University of Minnesota's Specimen Procurement (BioNet) facility.

Overall, I found the UROP experience to be extremely rewarding and beneficial to my education as an undergraduate biomedical engineering student. Going into this program, I was unsure how undergraduate research would affect me and my interest in my current program. However, I was immediately excited by the process of research and how different it is from any lab experience I have done through my coursework. I was able to work alongside passionate individuals who conduct research that changes the way we view and treat tumors. Because of this program, I now have incredible practical lab skills that I would not have gained otherwise. I learned how to do cell culture to produce MCTS, capture images of individual spheroids using

fluorescence microscopy, and use various image processing tools to analyze data. Beyond these technical skills, I have also learned how to work and communicate better with other individuals, such as my faculty mentors, to work more efficiently inside and outside of the lab. Without the assistance of the UROP program, I would not have been able to gain these valuable skills that I can use throughout the rest of my college career as well as in my life post-graduation.